RESTRICTION OF POLLINATOR ASSEMBLAGE THROUGH FLOWER LENGTH AND WIDTH IN THREE LONG-TONGUED HAWKMOTH–POLLINATED SPECIES OF *MANDEVILLA* (APOCYNACEAE, APOCYNOIDEAE)[1]

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ABSTRACT

Apocynaceae have a precise pollination mechanism. However, broad pollinator assemblages, including several insect orders, have often been recorded. We test how the corolla tube length and operative width required for effective release of the pollination mechanism could restrict the pollinator assemblage in putatively hawkmoth-pollinated Apocynaceae that differ in flower depth. If corolla tube length were effective in filtering pollinators, a broader pollen vector assemblage would be expected in short-flowered species. For this to occur, the operative width of the pollinating apparatus would be additionally expected not to be specific to any particular pollinator. Alternatively, if the pollination mechanism were to have any effect in the exclusion of pollinators, access to short flowers would be expected to be limited by proboscis width rather than length. Pollination of three species in the Apocynaceae was studied in seven populations through direct observations and palynological analysis of hawkmoths. Proboscis widths of hawkmoths carrying and not carrying pollen were compared with operative floral width as measured by proboscis analogues of different diameters that could successfully release the pollination mechanism. Species studied were exclusively hawkmoth pollinated. Pollen was always attached to pollinator proboscides, either near the base or on the apical half. The long-tongued hawkmoth species *Manduca sexta* (L.) was the major pollinator of *Mandevilla longiflora* (Desf.) Pichon and *Mandevilla petraea* (A. St.-Hil.) Pichon. Surprisingly, another long-tongued species, *Manduca tucumana* (Rothschild & Jordan), was the main pollinator of the short-flowered *Mandevilla laxa* (Ruiz & Pav.) Woodson. Here, the operative flower width was a decisive factor restricting the pollinator spectrum to hawkmoths with proboscides narrow enough to release the pollination apparatus. Short-tongued hawkmoths, which also have wider proboscides, cannot release the pollination mechanism. In *M. petraea*, the operative length, and not the operative width, restricts the pollinator assemblage. Thus, two different plant strategies were observed to restrict the pollinator spectrum: floral tube length and the operative width of the pollination mechanism.

Key words: Apocynaceae, long-tongued pollinators, *Mandevilla*, *Manduca*, operative flower width, plant-pollinator interaction, specialization, Sphingidae.

Floral structure in the family Apocynaceae received considerable attention from early botanists due to the elaborate pollination mechanism, consisting of a fertile organ complex in which anthers and stigma are coordinated or synorganized to place pollen packages precisely onto pollinators' bodies to achieve flower fertilization (Sprengel, 1793; Delpino, 1867; Endress, 1994). Most of this knowledge is based on observations of the pollination process in a few species and on broader inferences from the study of flower structure (Schick, 1980, 1982; Fallen, 1986). Although this family comprises a great number of species (ca. 4000 species) and is distributed worldwide, actual pollinator observations, i.e., visitors with pollen attached

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onto their bodies, have been reported for only approximately 120 species of Apocynaceae, indicating the need for more direct studies of pollination (Ollerton & Liede, 1997; Darrault & Schlindwein, 2005; Wiemer & Sérsic, 2006; APOPOL and ASCLEPOL databases).

The family now includes the previously recognized Asclepiadaceae (Endress & Bruyns, 2000; Potgieter & Albert, 2001), a large group composed of the subfamilies Periplocoideae, Secamonoideae, and Asclepiadoideae that is distinguished morphologically by important aspects of the construction of the pollination apparatus, which may be considered a further elaboration from the more basal subfamilies Rauvolfioideae and Apocynoideae (the Apocynaceae s. str.). Distinctive features of Asclepiadaceae include pollen dispersed in pollinia or tetrads that are provided with a translator composed of style-head secretions that have become solidified (P. Endress, 2004). In contrast, in Apocynaceae s. str. (now considered to be paraphyletic relative to the asclepiads: Potgieter & Albert, 2001; M. Endress, 2004) pollen is almost always in single grains, and the style-head secretions usually remain a viscid adhesive by which the mealy pollen adheres to the pollinators' proboscides. In this group, construction of the pollination apparatus has been studied in a considerable number of species (Demeter, 1922; Rowley, 1980; Schick, 1982; Fallen, 1986), but detailed accounts of the mechanical and dynamic aspects of its functioning have been analyzed more rarely (Demeter, 1922; Schick, 1980, 1982; Darrault & Schlindwein, 2005). Prior to and even after these studies, the dynamic aspects of the process were either incompletely or mistakenly interpreted. Schick (1982) centered his studies on the function of the pollination mechanism in two species, *Apocynum cannabinum* L. and *Vinca minor* L., which are visited (but not always pollinated) by a diverse array of insects. In this and other studies, it is not always clear whether high mechanical specificity of the floral mechanism serves to filter the sometimes broad array of visitors and narrows them to a more restricted group of actual pollinators.

In the pollination mechanism of Apocynaceae, especially members of the more derived subfamilies in which the anthers are postgenitally united with the style-head (Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae), one would expect that a considerable mechanical match is necessary between the dimensions of the pollination apparatus and those of the pollen vector, and thus, that only certain visitors with body structures mechanically suited to match the flowers can effectively function as pollinators. Interestingly, however, previous research on some Apocynaceae s. str. does not support this view, because both specialized and generalized pollinator assemblages have been observed involving pollinator species from one (Waddington, 1976; Haber, 1984; Bhatnagar, 1986; Ollerton et al., 2003; Oliveira et al., 2004) or several insect orders (Falcão & Lleras, 1981; Herrera, 1991; Ollerton et al., 2003). In addition, in a study on Asclepiadoideae, 126 species of pollen vectors were recorded on *Asclepias verticillata* L. (Ollerton & Liede, 1997).

Floral mechanisms in the Apocynaceae can be considerably versatile, and to some extent they limit utilization of floral rewards to certain visitors with suitable body geometry or behavior. For example, there are extensive records of flower visitors whose appendages become irreversibly lodged in the floral mechanism and are thus unable to pollinate (Romeo, 1933; and other references therein). This happens because of a mismatch between the width of the proboscis (or other appendage) and the width of the slit between the guide-rails (the lignified lateral sides of adjacent anthers). For such plants, the "operative width" of a proboscis can be thought of as a mechanism or strategy by which to narrow the pollinator spectrum.

In addition to operative width, a more common strategy used by Apocynaceae and many other flowering plants to narrow the pollinator spectrum has been the evolution of narrow, tubular corollas that limit flower visitors to those with mouthparts long enough to reach the nectar deep inside (Proctor et al., 1996). The most impressive of such examples are represented by plant species pollinated by long-tongued nemestrinid flies and extremely long-tongued hawkmoths (Darwin, 1862; Nilsson, 1988; Johnson & Steiner, 1997). This strategy has led to a close matching between the operative length of the flower and pollinator structures for imbibing nectar.

Within *Mandevilla* Lindl., the largest and most diverse genus of the tribe Mesechiteae, species with the sphingophilous syndrome (e.g., night-flowering, white corollas with slender lower tubes more than 10 cm long, and a strong, sweet scent) have evolved in parallel in the Northern Hemisphere (taxa previously included in the genus *Telosiphonia* (Woodson) J. Henrickson; Henrickson, 1996) and the Southern Hemisphere (taxa previously included in the genus *Macrosiphonia* Müll. Arg.; Ezcurra, 1981a, b; Galetto, 1997) and fall within two distinct clades (Simões et al., 2006, 2007a). In Argentina, four sphingophilous species with very long corolla tubes occur: *Mandevilla longiflora* (Desf.) Pichon, *M. petraea* (A. St.-Hil.) Pichon, *M. undulata* (C. Ezcurra) A. O. Simões, Kin.-Gouv. & M. E. Endress, and *M. virescens* (A. St.-Hil.) Pichon. In addition, *M. laxa* (Ruiz & Pav.) Woodson, with much shorter corolla tubes, also exhibits the sphingophilous syndrome (Ezcurra, 1981a, b). Pollination by hawkmoths has been recorded for *M. longiflora* (Silberbauer-Gottsberger & Gottsberger, 1975) and *M. petraea* (Galetto, 1997), but pollinator identities and details of the dynamic aspects of the pollination process have remained unknown to date. Diversity of hawkmoths is considerable in Argentina, where the family Sphingidae comprises 106 species, and the range of proboscis lengths varies from 13.3 to 246.1 mm. Each biogeographical region of Argentina generally includes assemblages of both short- and long-tongued species (Moré et al., 2005). Thus, we expected that the short flowers of *M. laxa* would be pollinated by both short- and long-tongued hawkmoth species, whereas the long flowers of *M. longiflora* and *M. petraea* would be pollinated only by long-tongued moths.

To determine pollinator specificity in *Mandevilla* and to explore the potential for the two mechanisms (i.e., operative tube length and width), we studied species with the same type of pollinating apparatus but contrasting flower lengths. Our focal species were three night-blooming, hawkmoth-pollinated species of *Mandevilla* native to Argentina: *M. longiflora* and *M. petraea*, which have very long corolla tubes (> 10 cm), and *M. laxa*, with much shorter corolla tubes (about 4 cm). If corolla length effectively filters pollinators, a broader assemblage of pollen vectors would be expected in the species with shorter flowers (Fig. 1). For this to occur, one would additionally expect that the operative width of the pollinating apparatus will not be adjusted to any particular pollinator. Alternatively, if the pollination mechanism has any effect in pollinator exclusion, one would expect that in short flowers visitors with proboscides too wide to release the mechanism would be excluded (Fig. 1). To test these predictions, we considered the following aspects: (a) pollinator species involved, (b) pattern of pollen deposition on their proboscides, (c) proboscis length and width, and (d) the efficacy by which the pollination mechanism is released by actual pollinators.

Materials and Methods

STUDY AREA

Flower measurements and pollinator observations were carried out in five populations of *Mandevilla petraea* (Cuesta Blanca, CB; El Durazno, ED; El Palmar National Park, EP; San Nicolás, SN; and Unquillo, UN), one population of *M. longiflora* (Bellavista, BV), and two populations of *M. laxa* (Santa Bárbara, SB; Cuesta Blanca, CB), all located in Northern Argentina (Table 1).

LIFE HISTORY OF THE SPECIES STUDIED

Mandevilla longiflora and *M. petraea* are rhizomatous perennial herbs that grow mainly on rocky soils in arid or semi-arid grasslands. Both species have long, salverform, vespertine flowers. The corolla comprises the lobes with strongly crinkled margins and a tube which can be divided into a narrow, cylindrical lower tube and an expanded funnel-shaped throat (Fig. 1). The length of the narrow, lower tube usually varies considerably between species (e.g., in Argentina), from 6 cm in *M. undulata* to up to 13 cm in *M. longiflora* (Ezcurra, 1981a, b). Flowers open at sunset and may last up to eight nights, as in *M. longiflora* (Silberbauer-Gottsberger & Gottsberger, 1975), remaining closed during the day. Nectar volume is up to 200 µl in *M. longiflora* (Silberbauer-Gottsberger & Gottsberger, 1975), and nectar is sucrose-rich with a mean concentration of 25% sucrose equivalents in *M. petraea* (Galetto, 1997).

Mandevilla laxa is a woody liana widely distributed in the tropical Andes from Peru to northwestern Argentina. In this species, flowers have much shorter tubes (< 4 cm long) than in *M. longiflora* and *M. petraea*. Flowers last several days, remaining open during the daytime (Ezcurra, 1981a). The nectar is sucrose-rich, and the mean concentration is 27.7% (Galetto, 1997).

Mandevilla petraea and *M. laxa* have white corollas with almost identical reflectance spectra (Moré et al., 2003). Both species also have similar chemical compositions of their flower fragrances (Sérsic et al., 2004), consistent with those of other hawkmoth-pollinated species (Knudsen & Tollsten, 1993; Raguso et al., 2003a, b).

FLOWERS

Height of the anther-cone within the corolla tube (measured from tube base to anther-cone tip) and length of the corolla tube (including both the narrow lower tube and the expanded throat) were measured to the nearest 0.01 mm using a digital caliper. Flowers of *Mandevilla laxa* were inspected for scales left by hawkmoth visitors by means of a hand lens (magnification 10×), a technique that has been shown to be effective elsewhere (Nilsson & Rabakonandrianina, 1988). Fruit set of flowers open to natural pollination was recorded in one population of *M. petraea* (CB) in two different years (2000, 2002).

POLLINATORS

Hawkmoth assemblages present in the studied populations (Table 1) were determined by sampling the insects using two vertical sheet light traps (Fry &

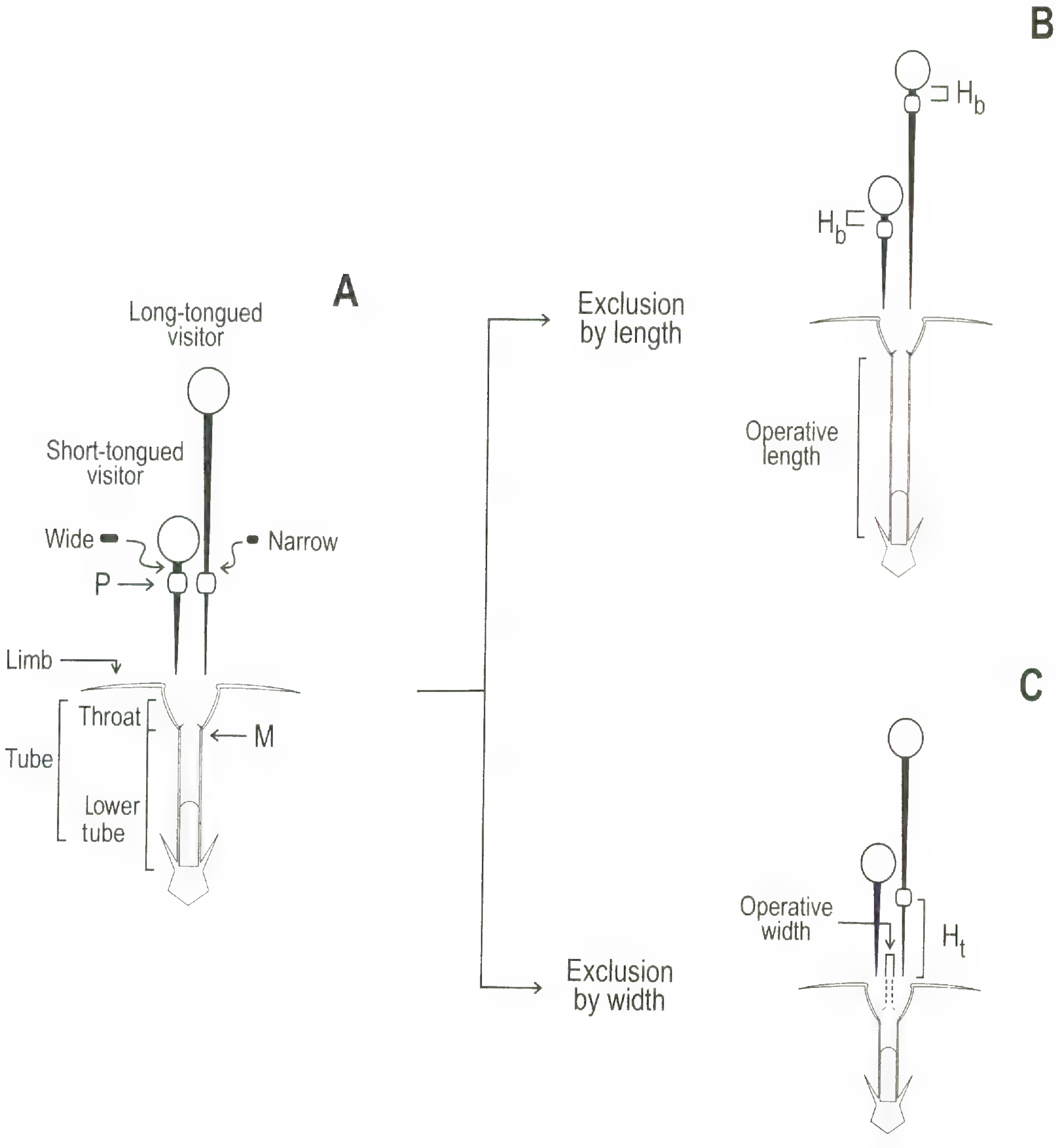

Figure 1. Hypothetical strategies for visitor exclusion in flowers of *Mandevilla*. —A. Ancestral flower type with a relatively short floral tube and a pollination mechanism (M) admitting both wide and narrow proboscides, from which short- as well as long-tongued visitors may take nectar and pollinate by extracting and depositing the pollen clumps (P), which become attached to their proboscides at a specific distance from the proboscis base (indicated as Hb in part B). —B. Hypothetical pathway leading to exclusion of visitors through increase of floral length. —C. Hypothetical pathway leading to restriction of pollinators based on the width of the pollination mechanism. Nectar is accessible to both short- and long-tongued visitors, but the entrance into the pollination mechanism is narrow, such that only visitors with a correspondingly thin proboscis are able to enter and function as a pollinator. Pollen clumps are deposited on the proboscis at a specific distance from the tip (Ht).

Waring, 2001) on a total of 14 nights. To determine the presence and location of *Mandevilla* pollen on the proboscides of the captured hawkmoths, specimens were inspected in the field under a Leica stereomicroscope (magnification 400×) (Leica Microsystems, Wetzlar, Germany). Pollen samples taken from clumps and from whole proboscides were mounted in glycerin jelly and identified under a light microscope using reference samples (Kislev et al., 1972). Direct observations of open flowers were carried out in the *M. petraea* populations totaling 22 nighttime and six daytime hours.

Proboscis length classes or guilds (irrespective of their taxonomic assignment) were assessed for the

Table 1. Populations of *Manderilla* species studied in northern Argentina and details of hawkmoth samplings. Codes refer to locations in northern Argentina: BV = Bellavista; CB = Cuesta Blanca; ED = El Durazno; EP = El Palmar National Park; SB = Santa Bárbara; SN = San Nicolás; UN = Unquillo.

Populations	Code	Vouchers	Province	Latitude S	Longitude W	Altitude (m)	Date of hawkmoth samplings	No. of captured hawkmoths	No. of hawkmoths carrying pollen of	
									M. longiflora or *M. petraea*	*M. laxa*
M. longiflora										
Bellavista	BV	*Cocucci et al. 2537* (CORD)	Corrientes	28°30′04.6″	59°02′55.5″	66	19 Nov. 2003	9	1	
							22 Feb. 2003	34	2	
M. petraea										
San Nicolás	SN		Córdoba	31°26′58.6″	64°25′40.1″	694	27 Nov. 2002	35	1	
Cuesta Blanca	CB	*Cocucci 3905* (CORD)	Córdoba	31°24′21.6″	64°34′00.5″	820	3 Nov. 2000	54	1	
							8 Nov. 2001	88	4	
							24 Oct. 2002	49	1	
							7 Jan. 2005	7	0	
							8 Jan. 2005	7	2	
El Durazno	ED	*Cocucci 3907* (CORD)	Córdoba	31°22′03.9″	64°37′41.8″	1117	4 Mar. 2001	15	2	
National Park El Palmar	EP	*Cocucci et al. 2954* (CORD)	Entre Ríos	31°52′29.6″	58°12′36.6″	159	16 Nov. 2003	3	1	
Unquillo	UN	*Benitez-Vieyra 3906* (CORD)	Córdoba	31°14′35.9″	64°18′03.8″	605	12 Feb. 2004	3	1	
M. laxa										
Santa Bárbara	SB	*Cocucci et al. 1360* (CORD)	Jujuy	24°15′52.5″	64°26′25.2″	1370	27 Nov. 2000	117		2
							22 Feb. 2001	78		20
Cuesta Blanca	CB	*Cocucci 3908* (CORD)	Córdoba	31°28′56.8″	64°34′34.7″	793	7 Jan. 2005			
Total								499	16	22

hawkmoths captured in the *Mandevilla longiflora* (BV), *M. petraea* (CB, ED, EP, SN, UN), and *M. laxa* (SB) populations. These were calculated by ordering specimens by proboscis length and then subtracting on a $\log_{10}$ scale the proboscis length of each individual from that of the previous shortest length. The greatest difference was used to split the data into two classes, and a Kruskal-Wallis test was performed to determine whether there were significant differences between these classes. This procedure was then iterated with the next greatest difference until no new significantly different groups were found.

We tested whether the proboscis length of captured hawkmoths influenced the probability of carrying pollen of *Mandevilla* species in each population. We thus determined whether hawkmoth individuals carrying pollen occurred in a random sequence in a series of increasing proboscis lengths by means of runs tests (Sokal & Rohlf, 2000). These tests were carried out for all hawkmoths captured in the populations of each plant species studied and separately for each guild of hawkmoths carrying pollen.

Differences in proboscis width were calculated for all hawkmoth species captured in the *Mandevilla petraea* and *M. laxa* populations. We expected that in the latter taxon (but not the former), floral mechanism rather than tube length would determine the specificity in pollen vectors. To detect differences in proboscis width, the insect guilds that contained individuals carrying *M. petraea* or *M. laxa* pollen were divided in two subgroups based on whether no individual of a species carried pollen or at least one individual of a species carried pollen. Proboscis width was measured in one to three specimens of each species of both subgroups at intervals of 5 mm distance from the proboscis base (Hb) for hawkmoths trapped in the *M. petraea* population, and from the proboscis tip (Ht) for hawkmoths trapped in the *M. laxa* population (Fig. 1). This was done because flowers are either longer (in the former) or shorter (in the latter) than the hawkmoth proboscides. Thus, the baseline from which to compare height of pollen deposition is respectively the pollinator's proboscis base or tip.

Measurements were made on digital photographs of the extended proboscides using the Image Tool program (Wilcox et al., 2002). Proboscis length guilds were tested with ANOVA for differences in proboscis width at each 5-mm interval of the $\log_{10}$ transformed width measures.

POLLINATION MECHANISM

Mechanical interactions between flowers and pollinators were studied from direct observations, photographs, videos, and the pollen deposition pattern on the proboscides of captured hawkmoths. In addition, two experiments using flowers of *Mandevilla laxa* and *M. petraea* were performed to determine: (1) the position along the model at which the pollen clump is attached, and (2) how the width of artificial proboscides might restrict the release of the mechanism.

In the first experiment, a straw (ca. 0.5 mm diam.) was inserted into the slit between the guide-rail of two adjacent anthers, down to the end of the corolla tube, in order to simulate a hawkmoth proboscis drinking nectar. The height on the straw at which pollen became attached during withdrawal was measured with a digital caliper. This experiment was performed in two populations of *Mandevilla petraea* (EP and UN, $n = 10$ and $n = 17$ trials, respectively) and on cultivated plants of *M. laxa* growing in the city of Córdoba ($n = 5$ trials).

The second experiment was performed on flowers of *Mandevilla laxa* ($n = 15$) and *M. petraea* ($n = 15$). A series of 10 fishing lines were introduced successively into the slit between the guide-rails in order of increasing diameter to test whether they successfully released the floral mechanism. A positive result was indicated by the pollination apparatus capturing the proboscis model. Fishing lines of 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, and 1.5 mm diameter were introduced once in each of two to five guide-rail slits per flower.

RESULTS

FLOWERS

Measurements of corolla tube length and height of the style-head in the corolla tube for the three species studied are shown in Table 2. *Mandevilla longiflora* and *M. petraea* had the longest flowers, whereas in *M. laxa* they were much shorter. Both floral measurements were significantly different in relation to species (ANOVA analysis: $F_{\text{tube}} = 283.51$, $P < 0.0001$; $F_{\text{style-head}} = 449.58$, $P < 0.0001$).

POLLINATORS OF *MANDEVILLA PETRAEA*

The 261 hawkmoths captured on the *Mandevilla petraea* populations belonged to 10 species (Tables 1, 3). There was a wide variation in proboscis length, ranging from 13.30 mm in *Callionima grisescens* (Rothschild) to 123.31 mm in *Manduca rustica* (Fabricius). These hawkmoth individuals could be classified into four insect guilds (Fig. 2A) that differ significantly in their mean proboscis lengths ($H = 218.52$; $P < 0.0001$): I short, 15.50 ± 1.31 mm ($n = 23$); II medium, 30.20 ± 2.34 mm ($n = 74$); III long,

Table 2. Mean ± standard deviation (s) corolla tube length (including both lower tube and upper throat) and height of the anther-cone within the corolla tube of the studied species of *Mandevilla*. n = number of individuals examined; for population codes see information in Table 1.

Species	Population	Flower measurements	Mean ± s (mm)	n
M. longiflora	BV	corolla tube length	118.92 ± 4.69	6
		anther-cone height	113.8 ± 4.55	6
M. petraea	CB	corolla tube length	105.95 ± 7.94	13
		anther-cone height	97.79 ± 7.82	13
M. laxa	SB	corolla tube length	44.18 ± 3.34	9
		anther-cone height	32.06 ± 2.28	9
M. laxa	cultivated	corolla tube length	27.43 ± 0.61	5
		anther-cone height	21.50 ± 0.94	5

55.30 ± 4.19 mm (n = 132); and IV very long, 100.44 ± 13.74 mm (n = 31).

Mandevilla petraea pollen was carried on the proboscis of only 13 hawkmoths belonging to four insect species: *Manduca sexta* (L.) (n = 10), *Manduca diffissa* (Butler) (n = 1), *Manduca rustica* (n = 1), and *Sphinx maura* Burmeister (n = 1). The mean proboscis length of these specimens was 92.53 ± 19.40 mm (Fig. 2A), of which the most frequent pollen vector, *M. sexta*, showed a mean length of 99.04 ± 8.10 mm. These specimens belonged to the long (n = 2) and very long (n = 11) guilds (III and IV, respectively). Thus, only long-tongued and very-long-tongued specimens were acting as pollen vectors for this plant. In Figure 2A, an aggregated sequence of pollen-carrying hawkmoths is evident in the very-long-tongued guild. The runs tests showed that in a series of all hawkmoths sorted by proboscis length, the sequence of those carrying *M. petraea* pollen was not random (observed runs of 18, expected runs at 25.70; $P < 0.0001$). However, sequences were random when the series were constructed separately from hawkmoths of guilds III and IV (guild III: observed runs of 7, expected runs at 6.86; $P < 0.7710$; guild IV: observed runs of 10, expected runs at 14.33; $P < 0.0550$). Thus, the insect individuals carrying pollen were aggregated in the ordered series of all hawkmoths captured.

Pollen of *Mandevilla petraea* was always attached to the proboscis base (Fig. 3E, H). The mean height at which pollen was attached was 85.23 ± 21.14 mm from the tip or 7.30 ± 5.37 mm from the base when all specimens were included, and 92.78 ± 11.22 mm from the tip or 7.40 ± 5.88 mm from the base for the *Manduca sexta* specimens alone (Fig. 2B). Two individuals of the long-tongued guild (*Manduca diffissa* and *Sphinx maura*) were found to carry *M. petraea* pollen and clearly represent outliers from the main sequence of pollen-carrying hawkmoths.

The subgroups of hawkmoths that were or were not carrying *Mandevilla petraea* pollen were significantly different in proboscis width at a height of between 5 and 35 mm from the proboscis base (Fig. 4A). In all cases, proboscides were wider in the subgroup of long-tongued and very-long-tongued species that were carrying pollen ($F_5 = 10.12$, $P < 0.0155$; $F_{10} = 5.37$, $P < 0.0537$; $F_{15} = 7.24$, $P < 0.0311$; $F_{20} = 7.09$, $P < 0.0374$; $F_{25} = 13.38$, $P < 0.0106$; $F_{30} = 11.16$, $P < 0.0156$; $F_{35} = 35.85$, $P < 0.0039$).

The fruit set of labeled flowers in the Cuesta Blanca population that were left to open pollination on November 2, 2000 (n = 65), and December 27, 2002 (n = 11), was 40% and 18%, respectively.

On only one occasion could visits by hawkmoths to *Mandevilla petraea* flowers be observed accurately. A single *Manduca sexta* individual was observed to completely introduce its proboscis into the flower and, while flapping continuously, settled on the corolla limb forcing the flower to swing toward the ground (Fig. 3G). A second individual of the same hawkmoth species was photographed in midair with a pollen clump attached to its proboscis' base (Fig. 3H). No other flower visitors were observed in the studied populations of *M. petraea*. During the daytime, flowers became flaccid, as the petal lobes folded back and the corolla tube drooped toward the ground.

POLLINATORS OF *MANDEVILLA LONGIFLORA*

The 43 hawkmoths captured on the *Mandevilla longiflora* population belonged to 13 species (Tables 1, 3). There was also a wide variation in proboscis length, ranging from 18.72 mm in *Callionima inuus* (Rothschild & Jordan) to 115.77 mm in *Manduca sexta*. These hawkmoths could be classified into two guilds (Fig. 5A) that differed significantly in their mean proboscis length ($H = 12.95$; $P < 0.0003$): I medium, 35.74 ± 11.47 mm (n = 38); and II very long, 107.15 ± 7.78 mm (n = 5).

Mandevilla longiflora pollen was carried by only three long-tongued specimens of *Manduca sexta* (mean proboscis length 112.60 ± 3.01 mm). The runs tests of all hawkmoths sorted by proboscis length

Table 3. Number of individuals with mean and standard deviation (*s*) proboscis length by species of all captured hawkmoths from the studied populations and of those carrying *Mandevilla* pollen. For population codes see information in Table 1. Ian J. Kitching (Department of Entomology, The Natural History Museum, London, United Kingdom) helped us with the identification of the hawkmoth species. All insect vouchers are deposited at the Laboratorio de Biología Floral, Instituto Multidisciplinario de Biología Vegetal (CONICET-UNCba), Córdoba, Argentina. Note: we were unable to measure the proboscis length in one specimen of *Manduca diffissa* from CB population.

Populations	Hawkmoth species	*n*	Mean (*s*) proboscis length of captured hawkmoths (mm)	*n*	Mean (*s*) proboscis length of specimens carrying *Mandevilla* pollen (mm)
M. petraea					
CB	*Callionima grisescens* (Rothschild, 1894)	15	15.81 (1.47)		
	Hyles euphorbiarum (Guérin-Méneville & Percheron, 1835)	2	27.53 (1.92)		
	Hyles lineata (Fabricius, 1775)	3	29.63 (3.84)		
	Manduca diffissa (Butler, 1871)	56	57.76 (6.87)	1	51.69
	Manduca rustica (Fabricius, 1775)	3	114.47 (7.89)	1	123.31
	Manduca sexta (L., 1763)	12	91.98 (15.27)	5	94.73 (4.6)
	Sphinx maura Burmeister, 1879	49	53.25 (3.14)	1	52.64
	Xylophanes tersa (L., 1771)	65	30.35 (2.18)		
ED	*Erinnyis ello* (L., 1758)	1	30.83		
	Manduca diffissa (Butler, 1871)	3	58.94 (4.44)		
	Manduca sexta (L., 1763)	9	108.04 (11.82)	2	108.6 (12.12)
	Xylophanes tersa (L., 1771)	2	28.58 (6.15)		
EP	*Eumorpha labruscae* (L., 1758)	1	47.14		
	Manduca sexta (L., 1763)	1	92.92	1	92.92
	Xylophanes tersa (L., 1771)	1	30.2		
SN	*Callionima grisescens* (Rothschild, 1894)	8	14.9 (0.65)		
	Manduca diffissa (Butler, 1871)	15	56.24 (2.93)		
	Manduca sexta (L., 1763)	2	98.65 (9.66)	1	105.48
	Sphinx maura Burmeister, 1879	10	54.44 (2.65)		
UN	*Manduca diffissa* (Butler, 1871)	1	59.65		
	Manduca sexta (L., 1763)	2	106.06 (6.89)	1	101.19
Total		260		13	
M. longiflora					
BV	*Adhemarius gannascus* (Stoll, 1790)	1	26.66		
	Callionima inuus (Rothschild & Jordan, 1903)	1	18.72		
	Erinnyis crameri (Schaus, 1898)	3	38.34 (1.92)		
	Erinnyis ello (L., 1758)	2	34.97 (0.8)		
	Erinnyis impunctata Rothschild & Jordan, 1903	1	37.56		
	Erinnyis obscura (Fabricius, 1775)	16	27.31 (1.77)		
	Eumorpha fasciatus (Sulzer, 1776)	4	45.54 (1.74)		
	Eumorpha labruscae (L., 1758)	5	56.72 (2.47)		
	Hyles euphorbiarum (Guérin-Méneville & Percheron, 1835)	2	29.3 (0.86)		
	Manduca florestan (Stoll, 1782)	1	59.54		
	Manduca sexta (L., 1763)	5	107.15 (7.78)	3	112.6 (3.01)
	Xylophanes pluto (Fabricius, 1777)	1	36.75		
	Xylophanes tersa (L., 1771)	1	32.66		
Total		43		3	
M. laxa					
SB	*Callionima grisescens* (Rothschild, 1894)	14	16.33 (0.78)		
	Erinnyis ello (L., 1758)	2	35.15 (2.14)		
	Erinnyis lassauxii (Boisduval, 1859)	2	41.74 (2.90)		
	Erinnyis obscura (Fabricius, 1775)	3	26.44 (1.57)		
	Erinnyis oenotrus (Cramer, 1780)	4	42.49 (1.08)		
	Eumorpha analis (Rothschild & Jordan, 1903)	1	43.28		

Table 3. Continued.

Populations	Hawkmoth species	n	Mean (s) proboscis length of captured hawkmoths (mm)	n	Mean (s) proboscis length of specimens carrying *Mandevilla* pollen (mm)
	Eumorpha neuburgeri (Rothschild & Jordan, 1903)	2	49.02 (0.68)		
	Manduca armatipes (Rothschild & Jordan, 1916)	4	47.37 (2.15)		
	Manduca bergi (Rothschild & Jordan, 1903)	18	56.65 (2.83)	1	56.45
	Manduca diffissa (Butler, 1871)	36	65.26 (5.60)	2	64.05 (2.06)
	Manduca sexta (L., 1763)	6	102.02 (4.52)		
	Manduca tucumana (Rothschild & Jordan, 1903)	52	78.84 (3.42)	18	78.69 (2.96)
	Sphinx aurigutta (Rothschild & Jordan, 1903)	4	55.63 (14.38)		
	Sphinx maura Burmeister, 1879	37	57.48 (4.41)	1	57.75
	Xylophanes pluto (Fabricius, 1777)	1	38.33		
	Xylophanes schreiteri Clark, 1923	6	35.57 (1.23)		
	Xylophanes tersa (L., 1771)	3	32.39 (3.44)		
Total		195		22	

showed that the sequence of those carrying *M. longiflora* pollen was not random (observed runs of 2; expected runs at 6.58; $P < 0.0001$). In Figure 5A, an aggregated sequence of the three pollen-carrying hawkmoths is evident in the very-long-tongued guild.

Pollen of this species also was attached to the base of the proboscides. The mean height at which pollen was attached was 99.59 ± 8.33 mm from the tip or 12.30 ± 7.06 mm from the base (Fig. 5B). One specimen was carrying two pollen clumps that were located at different proboscis heights (Fig. 5A).

POLLINATORS OF *MANDEVILLA LAXA*

The 195 specimens captured in the *Mandevilla laxa* population belonged to 17 hawkmoth species (Tables 1, 3). There was also a wide variation in proboscis length, ranging from 15.04 mm in *Callionima grisescens* to 106.99 mm in *Manduca sexta*. These hawkmoths could be classified into three guilds (Fig. 6A) that differed significantly in their mean proboscis length ($H = 53.70$; $P < 0.0001$): I short, 16.30 ± 0.78 mm ($n = 14$); II medium+long, 62.29 ± 14.44 mm ($n = 175$); and III very long, 102.02 ± 4.52 mm ($n = 6$).

Mandevilla laxa pollen was carried by 22 long-tongued individuals (Fig. 6A) belonging to four species: *Manduca bergi* (Rothschild & Jordan) ($n = 1$), *Manduca diffissa* ($n = 2$), *Manduca tucumana* (Rothschild & Jordan) ($n = 18$), and *Sphinx maura* ($n = 1$). These species belonged to the medium+long guild (II). The mean proboscis length of these specimens was 75.53 ± 7.59 mm. The runs tests showed that in the series of all hawkmoths, and of the medium+long guild (II) only, the sequence of pollen-carrying specimens was not random (all hawkmoths: observed runs of 29, expected runs at 40.04; $P < 0.0001$; guild II: observed runs of 29, expected runs at 39.47; $P < 0.0001$). In Figure 6A, an aggregated sequence of pollen-carrying hawkmoths is evident in the right half of the medium+long-tongued guild.

Pollen of *Mandevilla laxa* was attached near the distal half of the proboscides. The mean height at which pollen was attached on hawkmoth proboscides was 27.86 ± 3.93 mm ($n = 15$) from the tip (Fig. 6B). This height could not be measured in seven specimens because pollen passed unnoticed until all pollen found on the proboscis had been microscopically identified.

Of 135 flowers of *Mandevilla laxa* inspected in the Santa Bárbara population, only seven (5%) had scales left by hawkmoths. Only one diurnal visitor was observed on flowers at the Cuesta Blanca population. This was a halictid bee that could not release the mechanism and had its proboscis stuck in the slit between the guide-rail of two adjacent anthers.

Within guild II (medium+long proboscides), the hawkmoth subgroup that was not carrying pollen included the somewhat shorter-tongued species *Erinnyis ello* (L.), *E. lassauxii* (Boisduval), *E. oenotrus* (Cramer), *Eumorpha analis* (Rothschild & Jordan), *E. neuburgeri* (Rothschild & Jordan), *Manduca armatipes* (Rothschild & Jordan), *Sphinx aurigutta* (Rothschild & Jordan), *Xylophanes pluto* (Fabricius), *X. schreiteri* Clark, and *X. tersa* (L.). The hawkmoth subgroup with pollen-carrying individuals contained the longer-tongued species *Manduca bergi*, *M. diffissa*, *M. tucumana*, and *Sphinx maura*. The hawkmoth subgroups were significantly different in proboscis width at a height of 5 mm and between 20 and 40 mm from the proboscis tip (Fig. 7A). In all cases, proboscides were wider in the subgroup that did not carry pollen ($F_5 = 5.53$, $P < 0.0351$; $F_{20} = 8.62$, $P < 0.0116$; $F_{25} = 10.17$, $P < 0.0071$; $F_{30} = 6.69$, $P < 0.0238$;

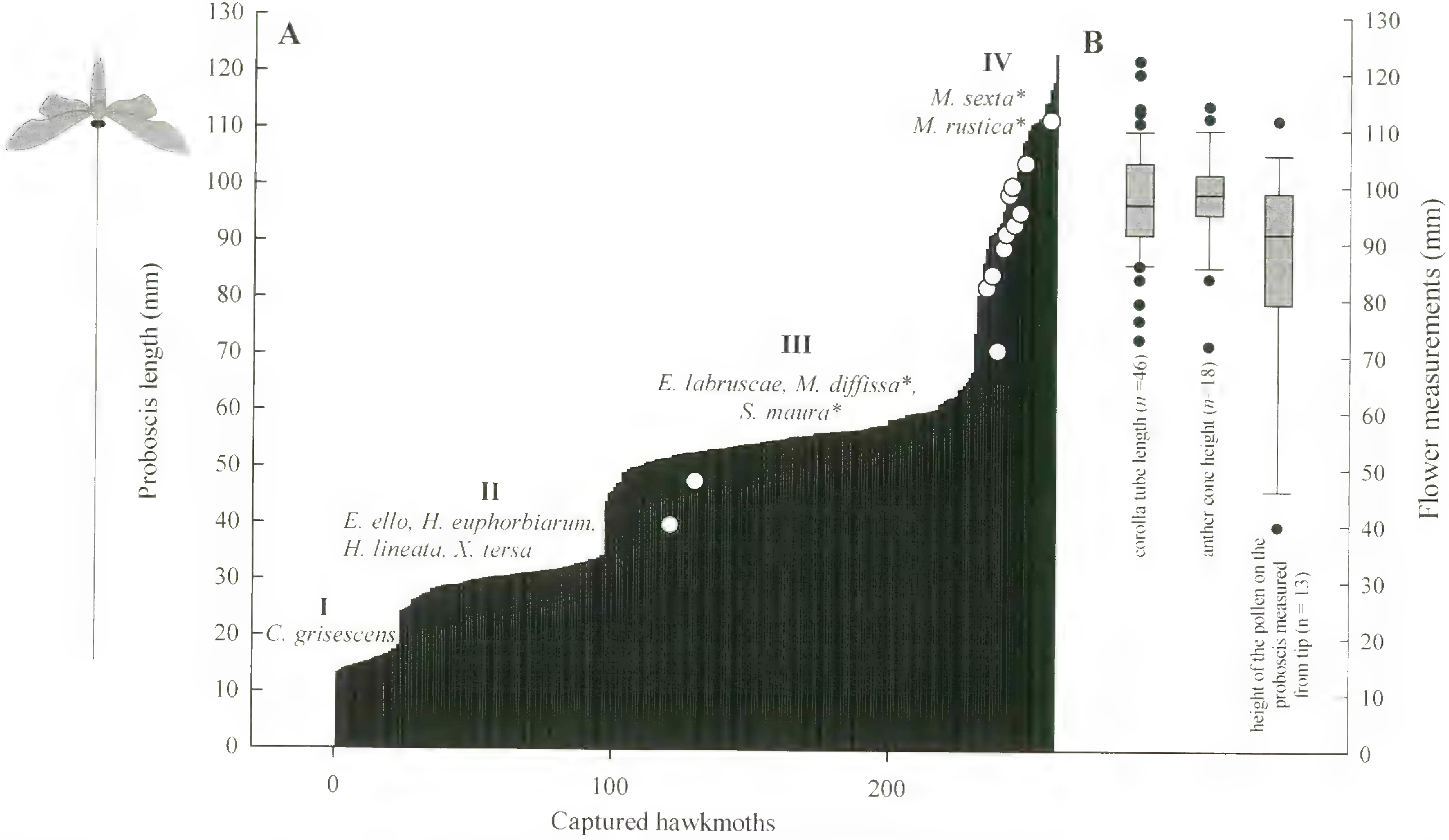

Figure 2. *Mandevilla petraea*. —A. Hawkmoth guilds, guild composition, and pollen vectors in five populations (CB, ED, EP, SN, and UN). Specimens sorted by increasing proboscis length. White circles show height on the proboscis measured from the tip at which *M. petraea* pollen was attached. Asterisks represent hawkmoth species carrying *M. petraea* pollen. —B. Flower depth, as corolla tube length, and operative length, as height of the anther-cone within the corolla tube and the height of pollen clumps on the hawkmoth proboscis measured from tip. Box and whisker plots show the 10, 25, 75, and 90 percentiles for data distribution.

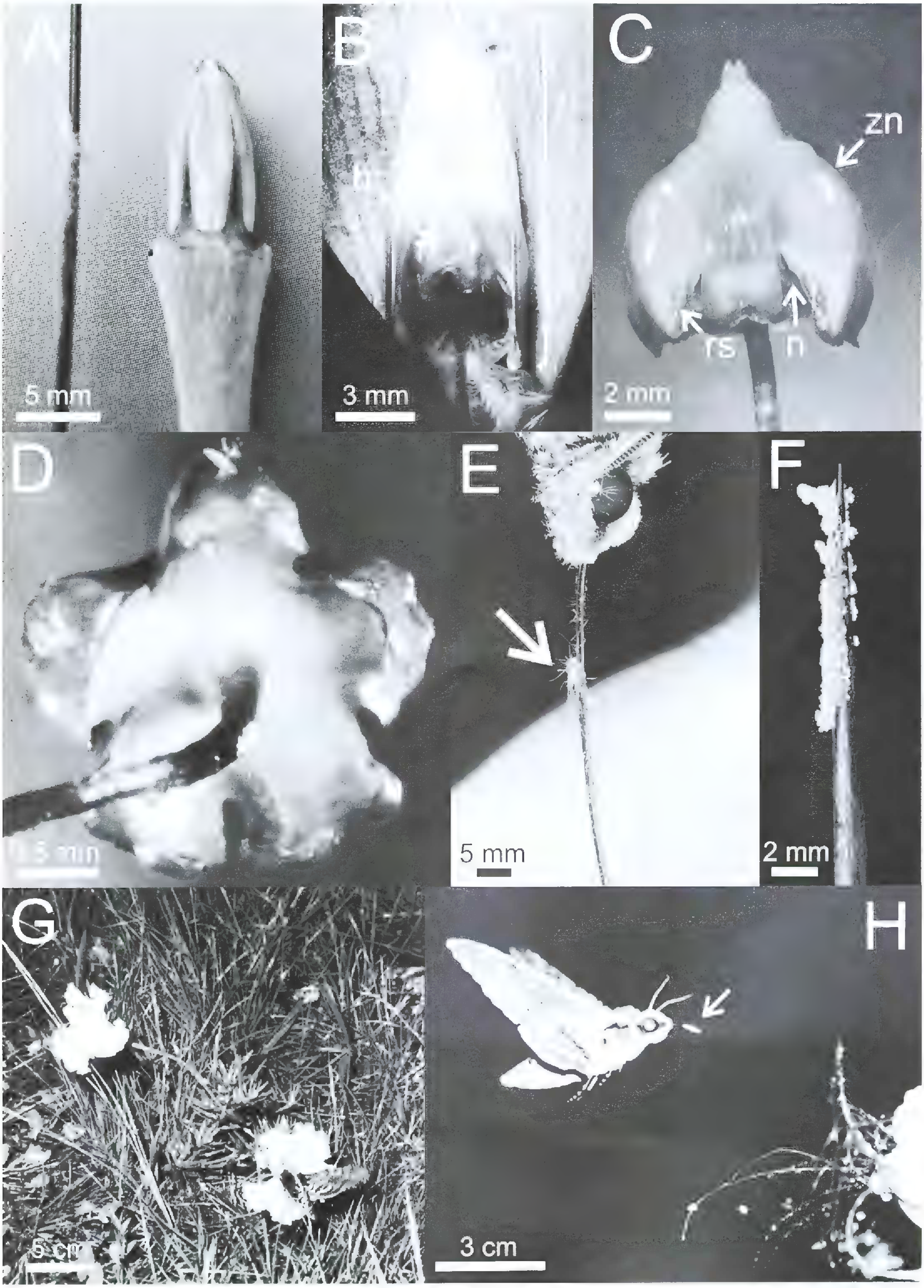

Figure 3. Floral mechanism and pollination in *Mandevilla petraea*. —A. Anther-cone situated at the top of the lower corolla tube, corolla throat removed. —B. Longitudinal dissection of the anther-cone showing the sterile (s) and fertile (f) parts of the anthers and the style-head (h). —C, D. Lateral and basal views, respectively, of the style-head showing the secretory zone (zn), notches (n), and receptive surface (rs). —E. Pollen of *M. petraea* on the proboscis base of a *Manduca sexta* male. —F. Pollen of *M. petraea* on the tip of a needle. —G. *Manduca sexta* visiting a *M. petraea* flower. —H. *Manduca sexta* visiting a *Caesalpinia gilliesii* flower (Fabaceae). Note the *M. petraea* pollen clump attached near the proboscis base (arrow).

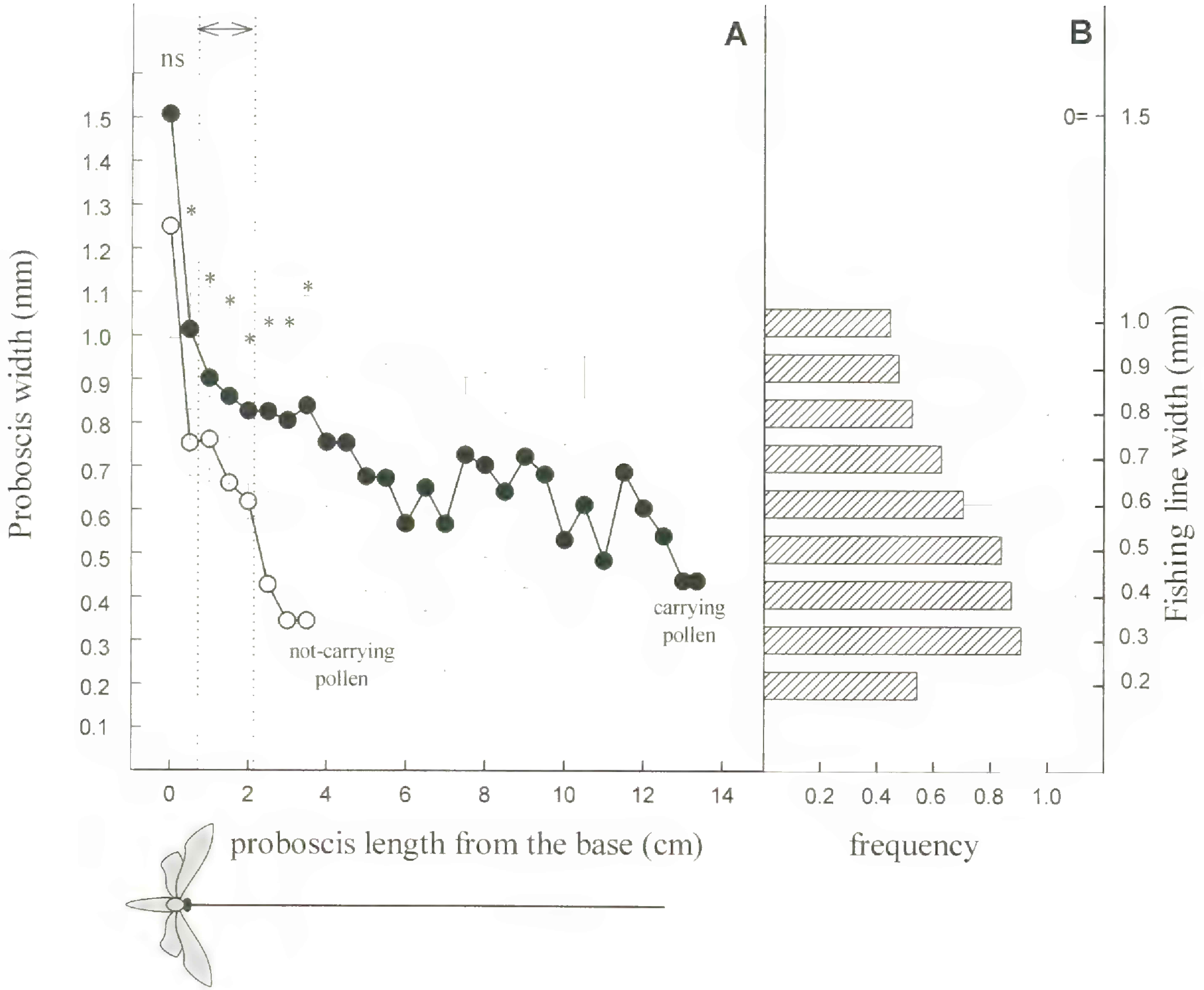

Figure 4. —A. Proboscis width measured from the proboscis base in short-tongued species that were not carrying *Mandevilla petraea* pollen (*Callionima grisescens*, *Hyles euphorbiarum*, *H. lineata*, *Erinnyis ello*, *Xylophanes tersa*, *X. schreiteri*, and *X. tersa*) and long-tongued species that were carrying *M. petraea* pollen (*M. diffissa*, *M. rustica*, *M. sexta*, and *S. maura*). The double-headed arrow at the graph top indicates the proboscis height interval in which *M. petraea* pollen was transported by long-tongued and very-long-tongued species. —B. Frequency of successful trials that released the pollination mechanism in *M. petraea* flowers. Fishing lines of 10 different diameters were used to simulate proboscides. Grey bars show mean successful trials, and error bars represent standard deviation (n = 13). Asterisks indicate significant differences in proboscis width between the subgroups of hawkmoths that were or were not carrying *M. petraea* pollen. See text for further details. "ns" indicates no significant differences in proboscis width between these subgroups.

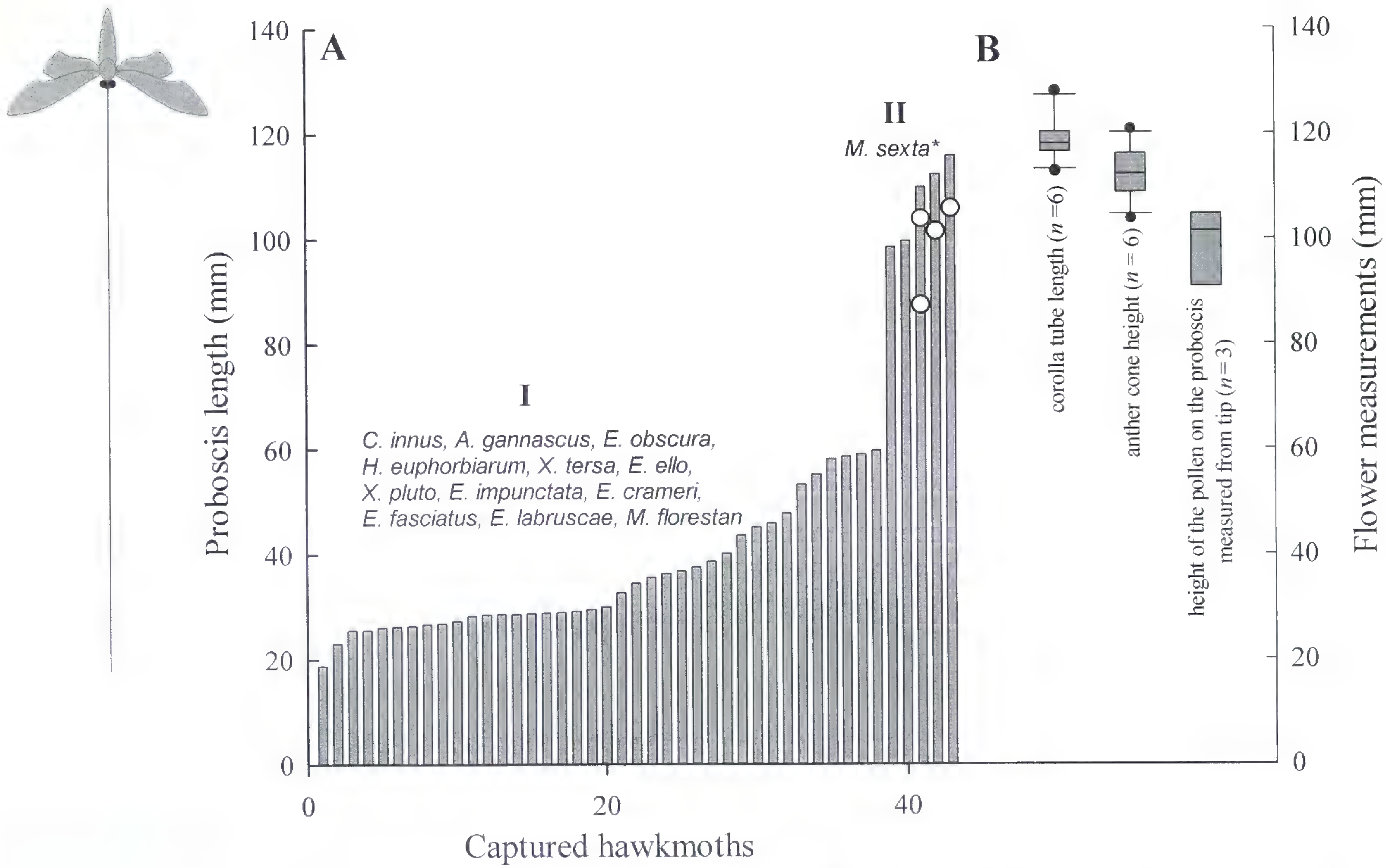

Figure 5. *Mandevilla longiflora.* —A. Hawkmoth guilds, guild composition, and pollen vectors in the Bella Vista population. Specimens sorted by increasing proboscis length. White circles show height on the proboscis measured from the tip at which *M. longiflora* pollen was attached. Asterisk indicates species carrying *M. longiflora* pollen. —B. Flower depth, as corolla tube length, and operative length, as height of anther-cone within the corolla tube and height of pollen clumps on the proboscis. Box and whisker plots show the 10, 25, 75, and 90 percentiles for the data distribution.

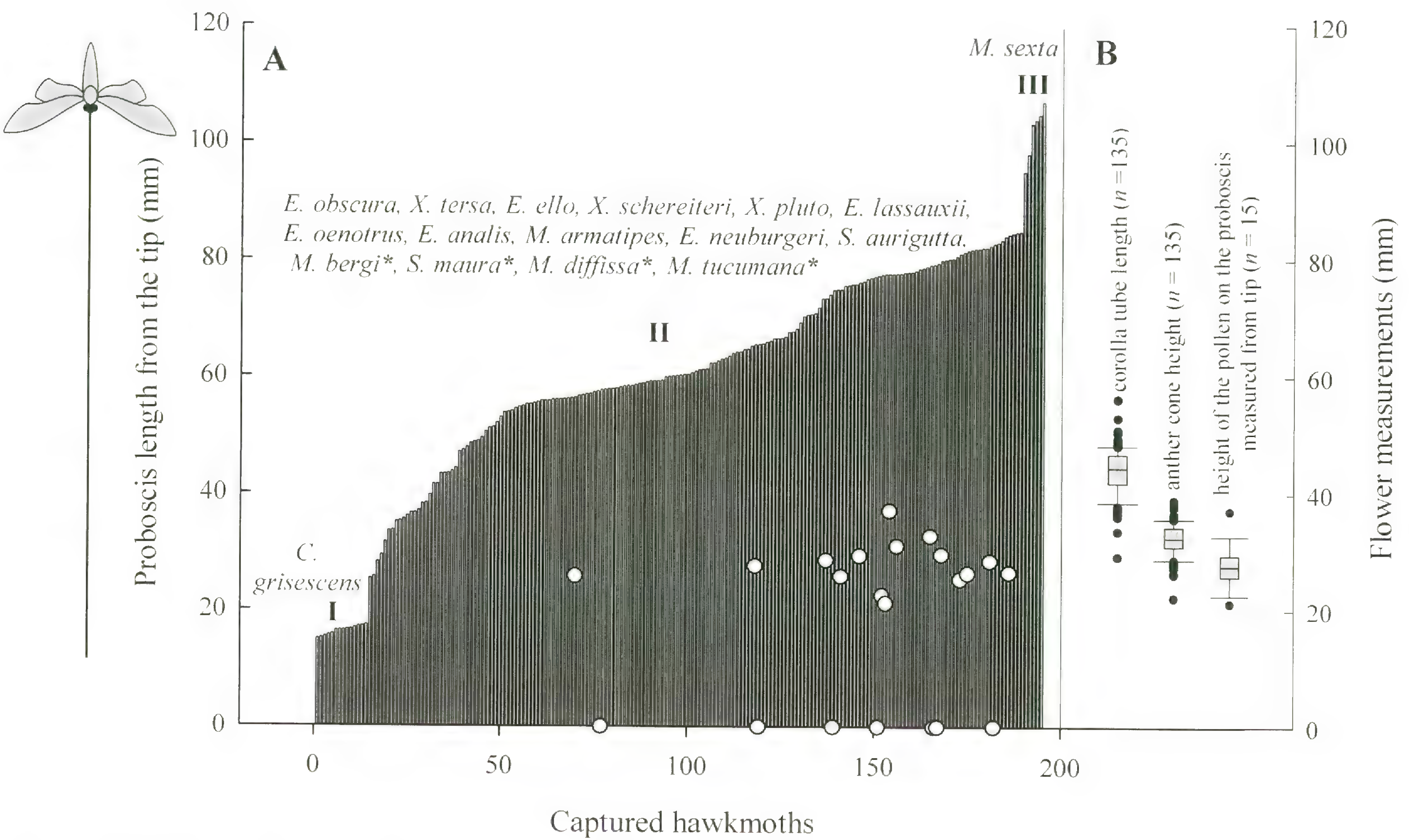

Figure 6. *Mandevilla laxa*. —A. Hawkmoth guilds, guild composition, and pollen vectors in the Santa Bárbara population. Specimens sorted by increasing proboscis length. White circles show height on the proboscis measured from the tip at which *M. laxa* pollen was attached. Asterisks indicate species carrying *M. laxa* pollen. —B. Flower depth, as corolla tube length, and operative length, as height of anther-cone within the corolla tube and height of pollen clumps on the proboscis. Box and whisker plots show the 10, 25, 75, and 90 percentiles for the data distribution.

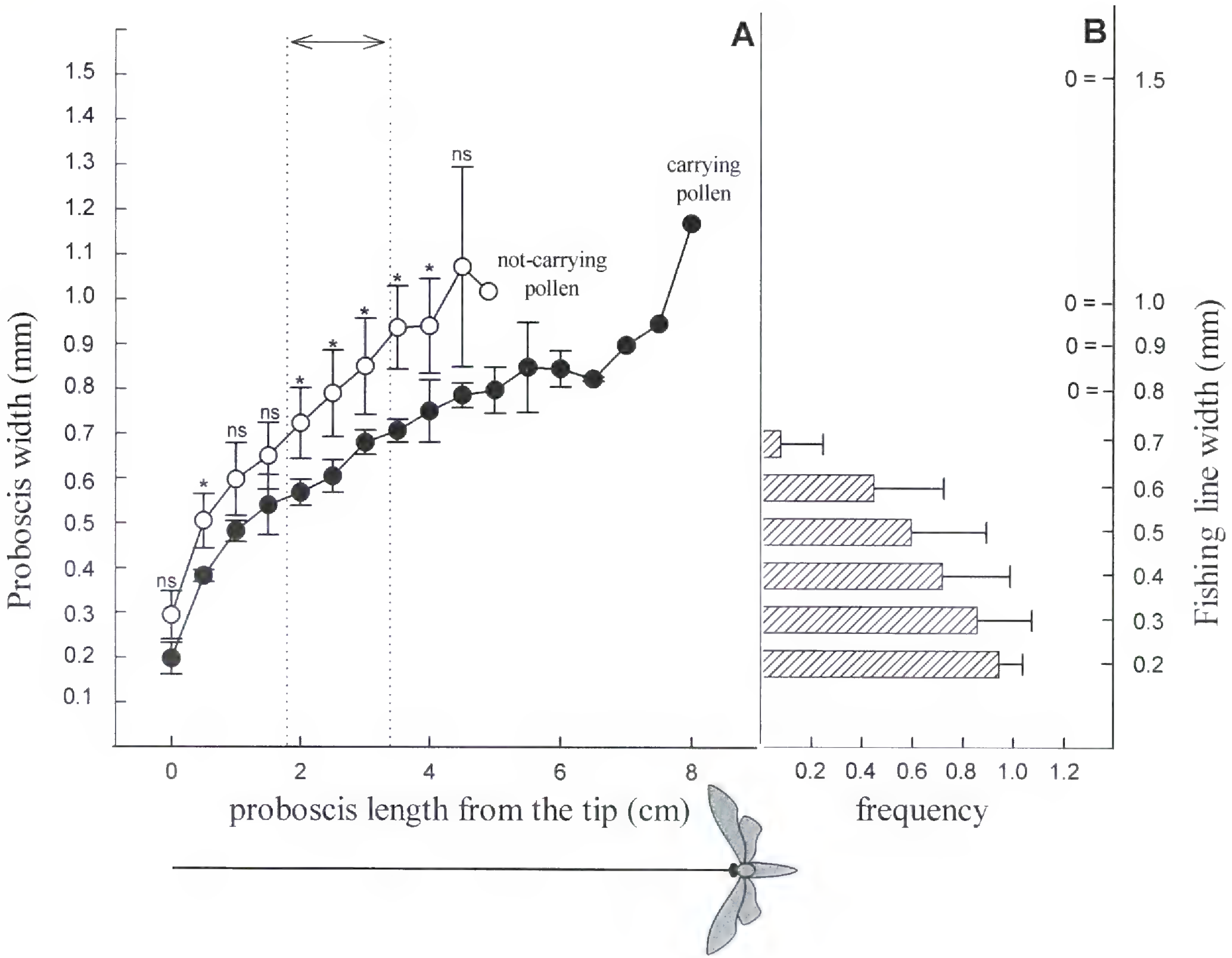

Figure 7. —A. Proboscis width measured from the tip toward the base in both short-tongued species that were not carrying *Manderilla laxa* pollen (*Erinnyis ello*, *E. lassauxii*, *E. obscura*, *E. oenotrus*, *Eumorpha analis*, *E. neuburgeri*, *Manduca armatipes*, *Sphinx aurigutta*, *Xylophanes pluto*, *X. schreiteri*, and *X. tersa*) and long-tongued species that were carrying *M. laxa* pollen (*M. bergi*, *M. diffissa*, *M. tucumana*, and *S. maura*). The double-headed arrow at graph top indicates the proboscis height interval in which *M. laxa* pollen clumps were transported by long-tongued hawkmoths. —B. Frequency of successful trials that released the pollination mechanism in *M. laxa* flowers. Fishing lines of 10 different diameters were used to simulate proboscides. Gray bars show mean successful trials, error bars represent standard deviation ($n = 14$). Asterisks indicate significant differences in proboscis width between the subgroups of hawkmoths that were or were not carrying *M. laxa* pollen. See text for further details. "ns" indicates no significant differences in proboscis width between these subgroups.

$F_{35} = 11.68$, $P < 0.0091$; $F_{40} = 7.48$, $P < 0.0291$). The largest difference between these subgroups was 0.197 mm (Fig. 7A).

POLLINATION MECHANISM

The construction and functioning of the floral pollination mechanism are essentially the same in all three species of *Mandevilla* studied. The anthers are united with the style-head, forming a complex organ, the gynostegium, located above the narrow lower corolla tube at the base of the expanded corolla throat. Anthers are lignified and connate, forming a sturdy anther-cone closely surrounding and solidly fused to the style-head. The boundaries between the guide-rails of adjacent anthers are represented by five longitudinal slits that are wider at the base and progressively narrow toward the apex, being completely closed along the apical 1/3 of the cone (Fig. 3A, B). Anther margins at the slits are sharp and curved inward. The anthers have a fertile apical part and a sterile basal part, the former corresponding to the closed region and the latter to the open region of the anther-cone slits (Fig. 3A, B). The style-head is completely enclosed within the lower part of the anther-cone and is radially differentiated, with five grooves alternating with the fused anthers, which are aligned and synorganized with the slits between the anthers, forming five longitudinal pollination chambers (Fig. 3C, D). The surface of the style-head produces an abundant slimy substance (the pollen transport adhesive; Fallen, 1986), which fills the lower region of the cone (Fig. 3B). The upper region of the cone above the style-head is radially divided into five longitudinal pollen storage chambers, each represented by the merged contents of the thecae of two adjacent anthers (Fig. 3B). Each channel opens at the tip through a soft, flexible anther acumen. The whole anther-cone is moveable by lateral tilting due to the flexibility of the short filaments subtending the anther-cone and to the thin style.

An artificial proboscis can be inserted into the corolla tube through any of the five inter-staminal spaces between the fused anthers. In so doing, the anther-cone is pushed and tilted aside. When the proboscis is withdrawn, it is caught by the base of the corresponding anther-cone slit. The inwardly curved anther slit margins act as a one-way trap, promoting upward and inward sliding along the slit through the pollination chamber. Upward traction of the proboscis opens the slits at the apical portion of the cone, which then closes back around the proboscis, sometimes with an audible click. Once the proboscis is threaded inside the pollination chamber, the anther-cone has returned to its original vertical position and the proboscis can be smoothly withdrawn. During withdrawal through the flexible anther appendages at the tip of the anther-cone, the proboscis successively slides along the concave stigmatic region beneath the expanded base of the style-head, the region filled with slimy pollen transport adhesive, and finally, the pollen chamber (Fig. 3B). Thus, pollen is attached onto the proboscis when it passes along each cone slit (Fig. 3A, F). If the proboscis was already carrying pollen, this is first deposited on the stigmatic concavity beneath the style-head.

The pollen on the hawkmoth proboscides was attached at a lower height from the tip than the height of the style-head within the corolla tube in all species. This difference, however, was significant only for *Mandevilla longiflora* and *M. laxa* (ANOVA analysis, $F_{laxa} = 34.43$, $P < 0.0001$; $F_{longiflora} = 11.83$, $P < 0.01$; $F_{petraea} = 3.45$, $P < 0.07$) (Figs. 3B, 5B, 6B). When artificial proboscides were inserted into the flowers of *M. petraea*, pollen became attached at a mean distance from the tip of 94.98 ± 6.74 mm ($n = 27$). This value did not differ significantly from that observed on the hawkmoths (ANOVA analysis, $F = 0.35$, $P < 0.71$) and was significantly lower than the height of the style-head within the corolla tube (ANOVA analysis, $F = 9.12$, $P < 0.004$). For the flowers from cultivated plants of *M. laxa*, the pollen was at a significantly lower height on the artificial proboscis 15.03 ± 0.87 mm than the style-head ($n = 5$) (ANOVA analysis, $F = 152.93$, $P < 0.0001$). This value was lower than the height of pollen on the proboscides of the hawkmoths from the Santa Bárbara population. However, we attribute this to differences between the native population and cultivated plants in height of the style-head in the corolla tube (Table 2).

In *Mandevilla petraea*, none of the trials with fishing lines 1.5 mm in diameter released the flower mechanism. Fishing lines 1 mm or thinner released the flower mechanism an average of 44%–90% of trials per flower (Fig. 4B). In *M. laxa*, none of the fishing lines thicker than 0.8 mm released the mechanism, whereas fishing lines 0.7 mm or thinner released the mechanism in 7%–94% times, and fishing lines 0.5 mm or thinner released the mechanism 60% of the time or more often (Fig. 7B).

DISCUSSION

The three *Mandevilla* species studied were pollinated exclusively by nocturnal hawkmoths. There are previous records of hawkmoth pollination for only nine species of Apocynaceae s.l., of which three belong to Asclepiadoideae (APOPOL and ASCLEPOL databases), *Cryptostegia madagascariensis* Bojer ex Decne., *Asclepias syriaca* L., and *Pergularia daemia*

(Forssk.) Chiov. Of the six species of Apocynaceae s. str., only two (*M. petraea* and *Plumeria rubra* L.) are known to be pollinated exclusively by nocturnal hawkmoths (Haber, 1984; Cocucci et al., 1992; Galetto, 1997). The four remaining species, *Carissa spinarum* L., *Hancornia speciosa* Gomes, *Nerium oleander* L., and *Vinca major* L., are pollinated by diurnal insects in addition to hawkmoths (APOPOL database: Darrault & Schlindwein, 2005). However, many putatively sphingophilous species occur within the Apocynaceae, and field observations are sorely lacking for most of them (Ollerton & Liede, 1997).

The present study demonstrates for the first time which hawkmoth species pollinate three *Mandevilla* species. Heterogeneity in flower form and color within *Mandevilla* suggests that many species do not attract hawkmoths. Indeed, bumble bees, euglossine bees, butterflies, and hummingbirds have been recorded as visitors to several species such as *M. hirsuta* (Rich.) K. Schum., *M. pentlandiana* (A. DC.) Woodson, and *M. rugellosa* (Rich.) L. Allorge (Kimsey, 1982; Torres & Galetto, 1998; Ollerton, pers. comm.). In contrast, the species that have a long corolla tube and open only at dusk, which were formerly treated as *Macrosiphonia* and *Telosiphonia*, appear to be exclusively sphingophilous (Ezcurra, 1981a, b; Henrickson, 1996), at least to the extent discernible by our examination of flower morphology, anthesis time, and pollen transfer mechanism.

Observed hawkmoth visits to the flowers of *Mandevilla petraea* were rare, even though 22 nighttime hours were dedicated to direct observation. Similarly, Silberbauer-Gottsberger and Gottsberger (1975) observed only one hawkmoth approaching the flowers of *M. longiflora* during five nights of observations. Despite this low visitation rate, the proportion of individuals setting fruit was relatively high (40% in 2000). Fruit set for *M. longiflora* also was observed to be relatively high by Silberbauer-Gottsberger and Gottsberger (1975). For *M. laxa*, visitation rate appears also to be low when estimated as the percentage of flowers with moth scales. However, this may be an underestimate of the actual visitation rate, because long-tongued hawkmoths may drink nectar without touching the corolla with their bodies.

The hawkmoths captured in populations of the plant species studied could be classified according to their proboscis length into three or four significantly different guilds. The three plant species of the present study utilized as pollen vectors only a fraction of the potential hawkmoth visitors available in the community. These hawkmoth pollinators were significantly confined in a narrow subset of all individuals sorted by proboscis length. Hawkmoths from the long-tongued and very-long-tongued guilds more often carried pollen of the short-flowered *Mandevilla laxa* and the long-flowered *M. longiflora* and *M. petraea*, respectively. The very-long-tongued *Manduca sexta* was the major pollinator (3 out of 3 in *M. longiflora* and 10 out of 13 in *M. petrea* of the pollen-carrying individuals) of both long-flowered *Mandevilla* species studied (Table 3). The long-tongued *Manduca tucumana* was the main pollinator (18 out of 22 of the pollen-carrying individuals) of *M. laxa*. The runs tests showed that within the long-tongued guild of hawkmoths visiting *M. laxa*, only the longer-tongued fraction was utilized as pollen vectors. This result was counter to our expectations and suggests that a flower mechanism other than corolla length is significant to the pollinator specificity in this species.

Apparently, through tube length alone, *Mandevilla longiflora* and *M. petraea* exclude most hawkmoths from the shorter-tongued guilds as pollinators. Pollen found on some individuals with much shorter tongues shows that they also can release the mechanism. However, their attempts to reach nectar are probably unsuccessful, which may attenuate the length of their foraging bouts or perhaps they may learn to avoid visiting these species. It was surprising to find that *M. laxa* used the long-tongued *Manduca tucumana* as its major pollinator, because its short flowers should have been accessible to the medium- and long-tongued hawkmoth guilds as well. Our data show that in this species, the operative flower width, rather than flower length per se, is the key factor in determining the type of hawkmoth pollinators that are used; thus, long-tongued pollinators are used even though flowers are relatively short. First, proboscis analogues more than 0.7 mm in diameter could not release the mechanism. Second, the mean proboscis width of short-tongued species not carrying pollen was above 0.7 mm along the segment between 20 and 40 mm from the tip and significantly wider than the long-tongued species that carried pollen. Third, pollen becomes attached to the proboscis within this range. Such a strategy, however, would not hinder some hawkmoths with short and wide proboscides to access nectar without pollinating the flowers. In conclusion, the moths with the longest tongues also had narrower tongues at the same distance from the tip than did the short-tongued species; so, those with proboscis lengths more comparable to the tube length of the flower were actually less suitable as pollinators because their tongues were too broad.

To our knowledge, only two other works have analyzed the importance of proboscis width in restricting pollen vectors. In the first, Nilsson and Rabakonandrianina (1988) hypothesized that the diameter of the spur cavity of the orchid *Aerangis* *ellisii* (B. S. Williams) Schltr. would prevent some hawkmoths from fully penetrating the flower. However, they failed to demonstrate this, because even the species with the broadest proboscis fitted fully into the floral spur. In the second, Darrault and Schlindwein (2005) hypothesized that the limited fruit production in *Hancornia speciosa* Gomes, an apocynaceous species pollinated by euglossine bees, butterflies, and hawkmoths, would be due to the different number of pollen grains removed by the proboscides of its different pollinator groups. They showed proboscis width is important not in restricting pollen vectors, but in the efficiency with which it can operate the pollination mechanism. Using proboscis analogues 0.3 mm in diameter or thicker, about twice as much pollen could be extracted from flowers of this species than when analogues were 0.2 mm thick. It is important to note, however, that *Hancornia* belongs to subfamily Rauvolfioideae, in which anthers are not postgenitally united with the style-head forming a gynostegium (Simões et al., 2007b), so that the movement and diameter of the proboscis are not as restricted as in members of subfamily Apocynoideae.

Among other plant families and plant-pollinator systems, evidence exists that operative flower width may be an important adaptive character in plants with much simpler flower mechanisms. Campbell (1996) found that flower width is under selection in the hummingbird-pollinated *Ipomopsis aggregata* (Pursh) V. E. Grant, in which wider flowers show greater male fitness by exporting greater pollen quantities per visit. The mechanical explanation of this pattern is that hummingbirds can insert their bills more deeply into wide flowers and that pollen removal is greater in deep insertions. However, this mechanism does not act to narrow the pollinator spectrum, and it is not actually adjusted to the width of any body part of the pollinator. Similarly, Galen (1989) found that a greater operative flower width is favored in an almost exclusively bumblebee-pollinated *Polemonium viscosum* Nutt. population. In another mostly fly-pollinated population of *P. viscosum*, a narrower operative length is presumably associated with selection for a wider array of smaller pollinators (Galen, 1989). In contrast to these examples, the width of the slit between adjacent guide-rails in *Mandevilla laxa* acts to restrict the use of certain pollinator species. Our data partially agree with observations made in other hummingbird-pollinated plants (Temeles, 1996; Temeles et al., 2002; Castellanos et al., 2004).

The three studied species of *Mandevilla* attach pollen onto hawkmoth proboscides in the form of aggregated clumps. Pollen of *M. longiflora* and *M. petraea* was carried on the base and that of *M. laxa* on the apical half of the proboscides. The proboscis width at these positions, which was greater for *M. petraea* pollinators, agrees with the operative width of the flower mechanism as measured with proboscis analogues of different diameters. Visitors with thinner proboscides may also be captured, and if these are not strong enough to release the mechanism, they may become trapped, as did one halictid solitary bee we observed in the Cuesta Blanca population. The species studied here all have a similar pollination mechanism, in which the insect's proboscis is guided into the opening at the base of the pollen chamber by the elongate basal appendages of the anthers and becomes threaded into the slit-like one-way trap by the lignified guide-rails (Schick, 1982). The mechanism requires the proboscis to slide a short distance along the slit for it to be captured. This explains why the pollen was always deposited at a position not equal to (Silberbauer-Gottsberger & Gottsberger, 1975), but significantly lower than the height of the style-head within the corolla tube. During withdrawal from the flower, once the proboscis is captured it cannot be pulled out at the base of the anther-cone and is forced to slide its way upward through one of the five pollination chambers. Once inserted in the slit between the guide-rails, the proboscis can be then gently slid out when the anther-cone tilts back. During the process of withdrawal, stigmatic concavity, pollen adhesive, and pollen chamber are passed in that order so that pollen may be deposited onto the receptive region, then the slimy pollen transport adhesive is smeared onto the proboscis, and finally pollen is glued onto it, as hypothesized by Schick (1980, 1982) and Fallen (1986). In the three species of *Mandevilla* studied here, the floral mechanism plays an important role in determining pollinator specificity.

At the opposite extreme, the 126 visitors carrying pollen of *Asclepias verticillata* recorded by Ollerton and Liede (1997) represent an assemblage of taxonomically diverse species that may share functionally equivalent pollinating structures making them suitable for operating the floral mechanism. These guilds constitute what have recently been called "functional groups," which are more relevant to specialization than are simple species lists (Armbruster et al., 2000; Fenster et al., 2004; Wilson et al., 2004).

In our study, the nocturnal hawkmoth community varied extensively in proboscis length and width, allowing us to recognize significantly distinct functional groups. Thus, having only a selected fraction of the available hawkmoths as pollinators may have been achieved by selective mechanisms that exclude functional insect groups with proboscides either too short or too wide to release the flower mechanism. This might represent an advantage for the plant's breeding success by reducing the chances of heterospecific pollen accumulation on the stigmas and pollen loss through incongruent deposition. Evolution of flower length has led to progressively longer operative lengths by excluding visitors with proboscides under-dimensioned for the flower length, as has already been shown in other studies (Johnson & Steiner, 1997; Nilsson, 1998).

Examples from the present study show that operative flower width excludes over-dimensioned visitors in *Mandevilla laxa*, and thus, evolution may have led to progressively thinner operative widths. Of those visitors whose proboscides are narrow enough, only those mechanically strong enough can function as pollinators, e.g., small bees were excluded as pollinators. Although we have studied only three species in the family, our study reveals novel insights toward the evolution of floral mechanisms that would be promising to address in future studies. Directional evolution of floral mechanisms in shorter-flowered *Mandevilla* and other Apocynaceae s.l. may have led the floral apparatus to adapt progressively to thinner pollinator parts, such as hairs on the insect legs or mouthparts of insects other than hawkmoths.

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